

Computerized Image Analysis for Determining Micronucleus Frequency

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A method for the computerized automation of micronucleus scoring is presented. The task is to identify the cultured, cytokinesis-blocked peripheral lymphocytes (CB cells) and their micronuclei (MN). The main parts of the hardware are the video camera attached to the microscope, the IBM-compatible personal computer with the video digitizer card, and the computer-controlled stage movement unit. The computerized image processing is based on determination and interpretation of contour lines of the CB cells, nuclei, and MN. The BNCTEST image processing software has been developed up to the demonstration phase, and now it has been prepared for the testing period of image series on a large scale.

Introduction

There are some different methods for examining physical and chemical effects (e.g., ionizing radiation, clastogenic compounds) causing chromosome aberrations. One of these methods is the examination of micronuclei (MN) in the injured cells, such as properly prepared cultured human lymphocytes (CB cells). This method (finding and scoring MN through a microscope) is much easier than, for example, the karyogram evaluation. Thus, it is possible to automate the image processing, avoiding the tiresome work of scoring. Image processing is performed by specially designed computers for image analysis (1) or by image processing systems based on personal computers (PCs) with appropriate hardware accessories [e.g., video digitizer cards and image processing boards (2-4)]. Our present work deals with the design and development of such a system.

Slide Preparation

The methods used to culture peripheral lymphocytes and to prepare slides for examination were published earlier (5,6). Staining was done with Giemsa solution, and as a result, gray-level images were obtained, and the objects differed from each other only in their densities (Fig. 1).

FIGURE 1. The Giemsa-stained image of a cultured and cytokinesis-blocked peripheral lymphocyte.

Hardware

The functions of the automated image processing system are to *a*) bring the image to the computer, *b*) visualize and evaluate the image, *c*) subsequently set further images. These functions are realized by the system shown in Figure 2. The image of the OPTON III RS microscope (1) is transformed to an analog video signal by the SDT-4500 black-and-white camera (3). The video signal is transmitted to the HRT 512-8 video digitizer card mounted on the IBM AT-286 compatible personal computer (5). This card makes an image of 512×512 pixel resolution for the computer with 256 gray levels for each pixel. The EGA monitor attached to the computer presents graphically that part of the image that is being processed, and the final results are presented numerically and also in

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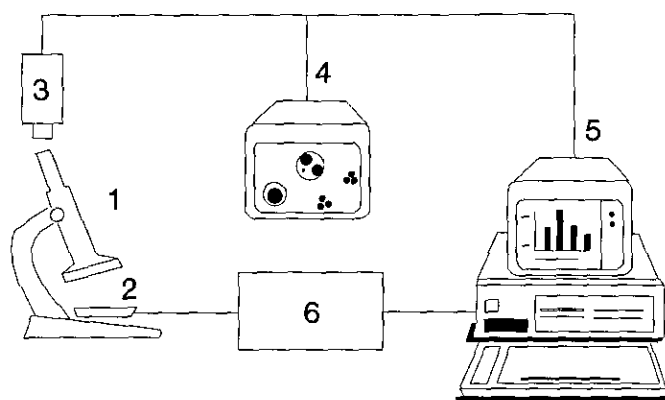


FIGURE 2. The hardware configuration of the image processing system.

a bar graph. The other monitor (4) shows the original image from the digitizer card via a digital/analog converter. The OPTON-type stage movement unit (2) is controlled by the PC through its parallel port and a power adapter (6), making it possible to scan the whole slide without human intervention. The present configuration does not allow the focus control by the PC; this must be done by the user after changing the slides.

Software Development

Image Processing Software

The image processing software BNCTEST recognizes the image of the CB cells and identifies and scores their MN and determines the micronucleus frequency from a sample containing 500 or 1000 CB cells. An additional option is collecting the area and density values of the identified nuclei and MN in a data file for further processing.

Identification of the CB Cells

We identify the CB cells by their densities, size parameters, and shapes as perceived by the camera. The CB cells are round objects, with characteristic measure range and tonality darker than the background. The nuclei and MN are darker than the cell plasm, they have also a round shape, may appear alone, and may contact or partially overlap each other. The nuclei have about the same measures and densities, the measures of the MN are in a smaller range, and the densities are between that of the cell and the nucleus, depending on their DNA content.

Determination of the Threshold Values

After inserting the new slide, the image processing starts with the determination of two density threshold values. The pixels, brighter than the first threshold level (cell plasm level, t_p) form the image background. Those that are darker than the second threshold level (the nucleus level, t_n) belong to the nuclei or MN. The pixels with densities between these two levels constitute the cell plasm. The nucleus level is determined as described (?): by finding the density value minimizing entropy made up

from the gray-level frequencies in the image (Fig. 3). The cell plasm level is determined as follows:

$$t_p = t_n + (h_1 - h_2)/3,$$

where h_1 is the gray value of the darkest pixel and h_2 is that of the brightest one. The factor 1/3 is an empirical value.

Scanning

To accelerate the processing, we apply a presearch procedure, by fast scanning the image, searching for objects with measures corresponding to a CB cell. If the side measures of its enframing rectangle (Fig. 4) are in the preset range, the coordinates of the rectangle will be stored. Further processing is made within these windows, instead of the whole image. This method accelerates the processing more than 10 times. This procedure rejects the cells touching each other. The evaluation of these cells might lead to false conclusions, but ignoring them does not influence the statistical result.

Determination of Contour Lines

Following the presearch procedure, the image processing is continued with the determination and examination of the contour lines of cells, nuclei, and MN. As a first step, the cell plasm contour is determined in the next image field, getting the coordinate series of the contour line (Fig. 5). This is done in every image field first for the cell plasm level and after for the nucleus level, but only within the presearched windows.

Identification of the Cell Plasm of CB Cells

After producing the contour line of the cell plasm, it must be verified that the line really represents a CB cell. The examination proceeds in several steps. If a given object does not satisfy a criterion, it is promptly rejected, and the examination continues with the next object.

The sides of the windows and the area of the objects must be in a preset range. The circularity is checked by the ratio of the radius determined from area and the maximum distance of the center and the contour line. If these values correspond to those of the preset ones, the object is considered a CB cell.

Identification of Nuclei and MN

Having found a CB cell, we produce the contour lines of the nuclei and MN and we have to examine each: The object (its center) must be within the cell plasm contour. It is rejected as a false micronucleus caused by the density fluctuation if its area is below an empirical minimal value. We search and divide the touching round objects (Fig. 6). After dividing, we check the shapes of the fragments.

All these manipulations are done on the two vectors of the coordinate series by transposing, inserting, and combining their parts. All the objects within the cell plasm are examined, even those produced by dividing or separation.

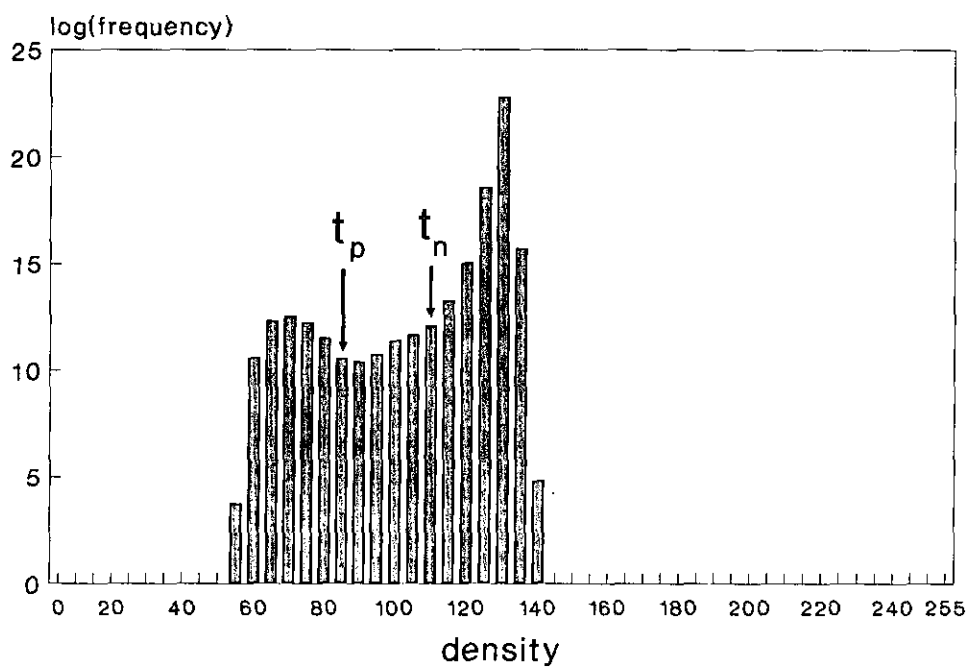


FIGURE 3. The histogram of the gray-level frequencies of the image.

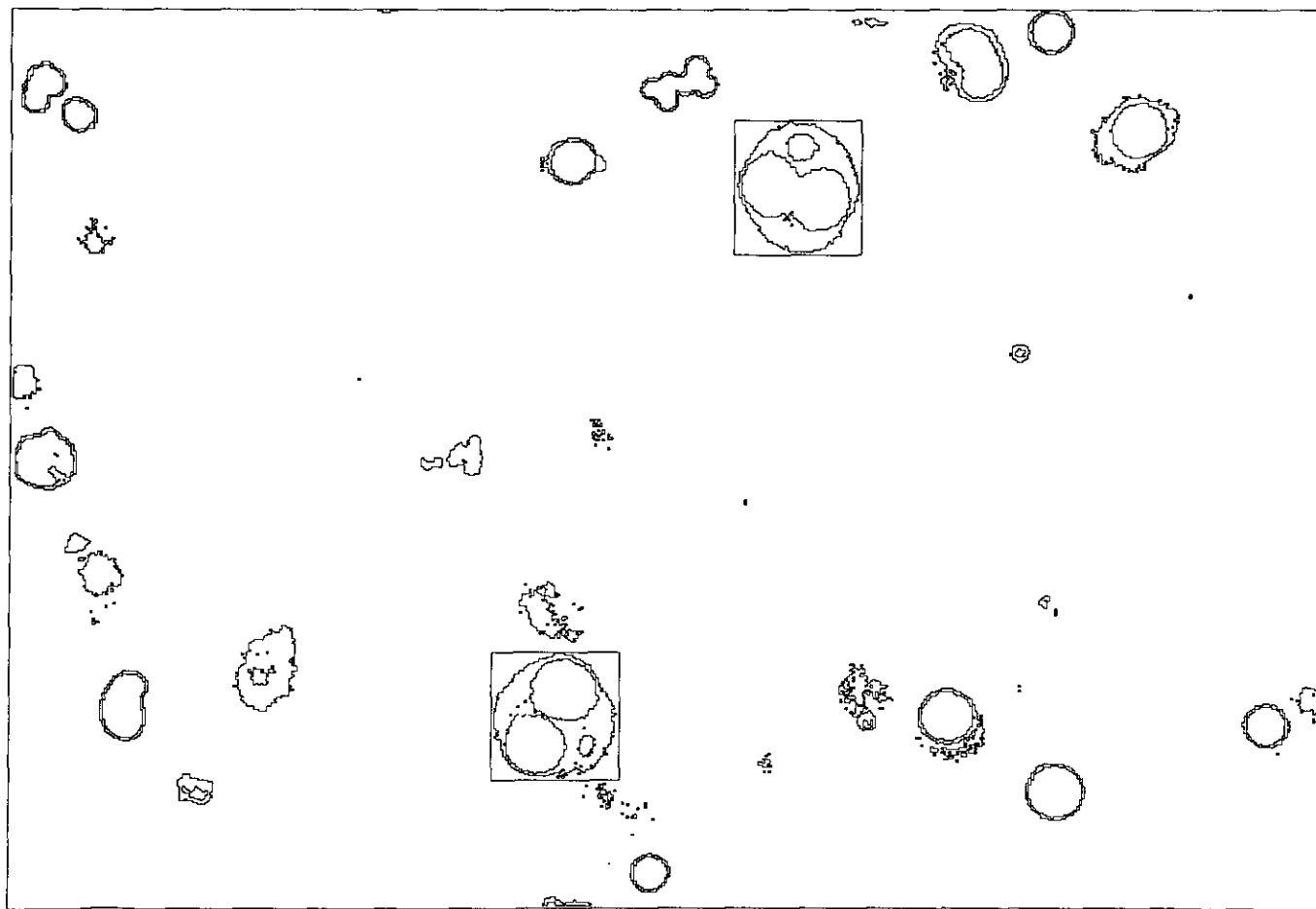


FIGURE 4. The presearched windows of the image, containing objects that are expected to be cytokinesis-blocked cells.

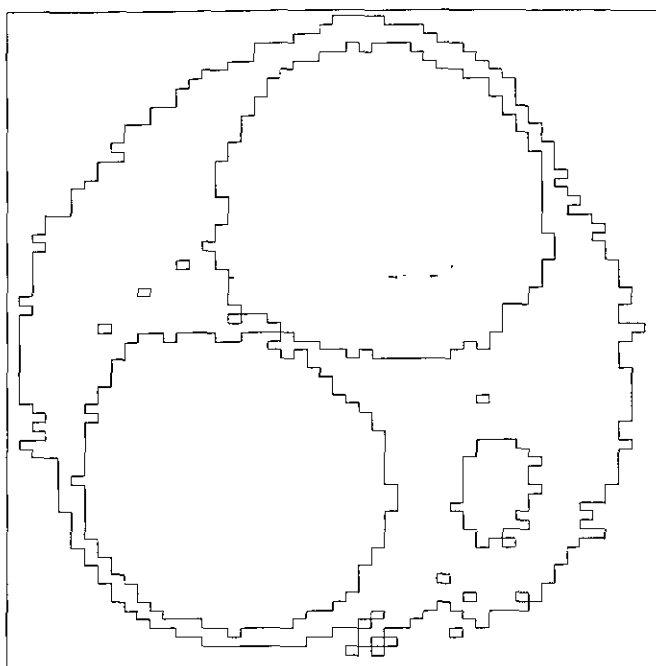


FIGURE 5. The image of a cytokinesis-blocked cell, made up of contour lines.

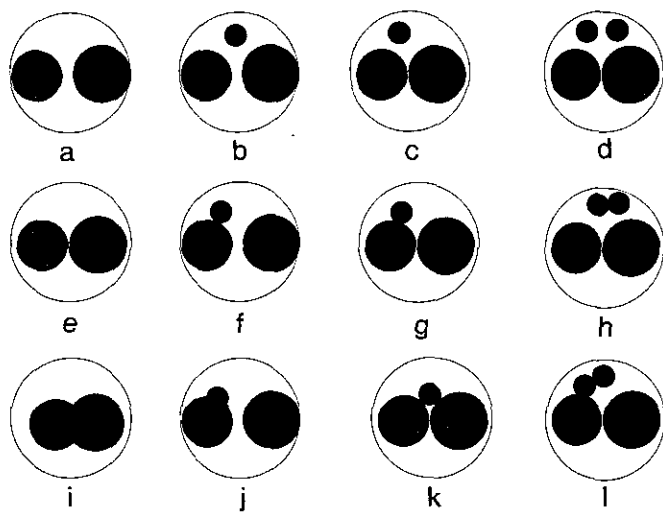


FIGURE 6. Cases of connections of two nuclei (a,e,i), one nucleus and one micronucleus (MN) (b,f,j), two nuclei and one MN (c,g,k), and two nuclei and two MN (d,h,l).

Every object that is not considered to be combined by its measure and shape is classified as nucleus or micronucleus. If there are two nuclei in a cell, it is regarded as a CB cell, and the number and other data of the MN are stored. Then processing continues with the next cell. After the last cell has processed in the image field, the next stage starts and the scanning follows again.

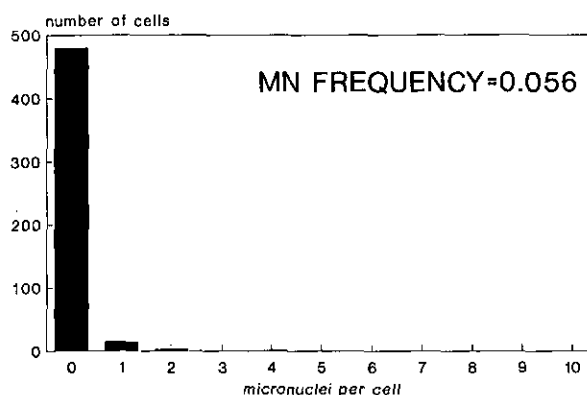


FIGURE 7. Acquisition the result of a micronucleus frequency analysis.

Display of the Processing and Results

The coordinate manipulations presented above are displayed on the EGA monitor of the PC. In the windows assumed to contain a CB cell, the contour lines are drawn in red. If it proves to be a CB cell by the examination, its lines are repainted. As the processing is fairly quick, the changes appear to blink, and one can check only the final picture.

When the preset number of CB cells is reached, the final result is presented by the micronucleus frequency value per 1000 based on scoring 500 or 1000 CB cells and also in a bar graph representing the frequencies of the multiple-micronucleated CB cells (Fig. 7). The measure and density values are stored in a data file.

Conclusion

The first period in development of the BNCTEST code has finished. It proved to be suitable for automatic determination of the micronucleus frequency. It can recognize the larger part of the probable images, including nuclei touching or overlapping each other. The next task is to make a test series on a large amount of pictures to find errors and to determine the reliability of the system.

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